

Acute Toxicity Among Greenhouse Farmers In Gaza Strip

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Summary: Greenhouse technology intensively increased in Gaza Strip and used large amounts of insecticides and fungicides for pest control. This created toxicity symptoms among farmers. This study characterized the acute toxicity of applied pesticides among farmers working in greenhouses. The study based on data collection from 57 greenhouse farmers, blood collection for acetylcholine esterase determination, and health records investigation of the acute cases. It appeared that about 139 farmers were working in greenhouses for tomato and cucumber production and spraying pesticides several times during the week. Results documented nine insecticides and five fungicides applied for insect and fungal control. Farmers revealed that they did not comply with pesticide safety measures during work. The following cholinergic symptoms such as convulsions, tremors, dizziness, headache, nozai, and diarrhea were associated with severe reduction on AChE activity. Serum blood analysis indicated sever inhibition of acetylcholine esterase activities (AChE) and elevated levels of ALT, AST and uric acid. Clinical investigation showed pin point pupil's, sweaty and salivation associated with sever AChE inhibition. Treatments showed that gastric lavage, good ventilation and atropine application provide good management with some cases. It is recommended to comply with pesticides safely measures of pesticide application in greenhouse to avoid intoxication.

Key words: Insecticides, acetylcholine esterase, acute toxicity, ALT

I. Introduction

Greenhouse technology progressively increased in Gaza Strip to meet the population needs from vegetables and ornamentals. It employs large number of farmers for several agricultural activities such as pesticides applications. So far pesticides application in greenhouse progressively increased due to intensive agriculture and caused many environmental problems (Schechter et al., 1997 a,b; Safi et al., 2002; El-Nahhal 2004). Health hazards have been documented with pesticides application such as cancer cases (Safi et al., 1993) health disabilities (El-Nahhal and Radwan 2013) and biochemical changes (El-Nahhal 2016). Exposure to pesticides may occur via indirect way such as drinking of contaminated water (El-Nahhal 2006, El-Nahhal and Hararah 2013) ingestion of contaminated food (Safi et al., 2002), working with soil components (Heinze et al., 2014) and contaminated air (Bornstein, 2001).

Pesticide residues are present as mixtures in the environment and may cause synergistic or antagonistic effects that alter the balance of human beings and/or an ecosystems (Wendt, et al., 2004). Ecotoxicity of pesticides have been reported to cyanobacteria (El-Nahhal et al., 2013; Safi et al., 2014; El-Nahhal et al., 2015a; Ma et al., 2010), to plant (El-Nahhal and Hamdona 2015)) to fish (El-Nahhal et al., 2015b; El-Nahhal and Al-Dahduh 2015).

Several attempts have been made to reduce contamination and exposure to pesticides, this includes development of ecologically acceptable organo-clay formulations of pesticides (El-Nahhal et al., 1997, Rubin et al., 2001, El-Nahhal 2003a,b,c, El-Nahhal and Safi 2004, 2005, El-Nahhal et al., 2005, Nir et al., 2000, 2006). Acute toxicity among farmers may be appeared due to occupational and/or accidental exposure to direct insecticide solution. In addition, misuse or un-comply with safety measures of insecticides may lead to acute toxicity appearance among farmers. So far, acute toxicity among farmers was indicated by the measuring the toxicity symptoms or activity of acetylcholine esterase in blood serum. For instance, García-García et al., (2016) indicated that erythrocyte acetylcholinesterase was significantly decreased in greenhouse workers relative to control sample (non-farm workers) due to occupational pesticide exposure. In a different study Ahmad et al., (2016) revealed that Bendiocarp, carbofuran, carbaryl, methomyl and propoxur significantly lowered the AChE activity greater than 50% due to accidental exposure to pesticides. In addition Cable and Doherty (1999) found acute carbamate and organochlorine toxicity causing convulsions in an agricultural pilot accidentally exposed to pesticide solution.

Similarly, Araoud et al., (2016) found methamidophos-treated groups had significantly lower butyrylcholinesterase and paraoxonase activities. Moreover, occupational exposure to pesticides has been documented in many countries around the globe (Mecdad et al., 2011; Faria et al., 2014; Galea et al., 2015; Bergkvist et al., 2012; Kim et al., 2015). These reports described knowledge, practices and attitude of farmers and lack acute toxicity determination and biochemical changes among farmers working in greenhouses of tomato cucumber and melon. So far, it is necessary to characterize the acute toxicity of pesticides under greenhouse conditions to be able to minimize and/or control the possible health hazards. Accordingly, this study

was initiated to answer the following questions: (1) what are the acute toxicity symptoms among farmers applying insecticides and fungicides in greenhouses (2) what are magnitude of toxicity parameters among farmers and (3) does application of pesticides mixture affect toxicity status among farmers in Gaza.

II. Materials and Methods

1. Data collection:

About 57 greenhouses were visited for data collection and 139 farm workers were interviewed in the field via direct contact, face to face interview. The data collection included occupational parameters related pesticide knowledge, toxicity symptoms and protective measures related to pesticide application in greenhouse.

2. Blood sampling and analysis for enzyme activities

Farmers working in greenhouse were invited to join workshop entitled “pesticides toxicity to non-target organisms”. Then, they were invited for voluntarily blood donation of 10 ml in a private clinic. Blood samples were collected in heparin containing tubes and analyzed in the same day for Acetylcholine esterase activity (ACHE), ALP, AST activities and uric acid concentrations. For instance ACHE was measured by a spectrophotometer according to the method developed previously by Ellman et al. (1961) the activity of AST and ALT activities were measured by the procedure of Reitman and Frankel (1957) using a commercially available kit from Bio-Merieux.

3. Analysis of personal health records of farmers

Personal health records of farmers having sever or acute toxicity symptoms were collected and carefully summarized.

4. Statistical Analysis

The collected data were summarized in response to locations. Insecticides and fungicides applied in greenhouse were collected along with their properties and presented in a separate table. Moreover, the data of personal health records were also presented in two separate tables. Toxicity symptoms were arranged according to locations, total and percentage values of symptoms were calculated. Toxicity among farmers was categorized into five groups according to the activity of Acetyl cholinesterase. These five groups are SI = Sever inhibition; I= Inhibition; MI= moderate inhibition; SLI= Slight inhibition; LI = less inhibition and NR = normal range. Moreover, correlation between uric acid, AST and ALT levels among farmer having different symptoms were also demonstrated, number of cases and their percentage among all were calculated.

III. Results

3.1 Pesticide information

Table 1 shows distribution of greenhouses and working farmers and under investigation in Gaza Strip. It appeared that 139 farmers were the manpower in 57 greenhouse located in different governorates in Gaza Strip. Moreover, about 18 non-farmer individuals were referred to as control samples. So far, the most frequent pesticides used in greenhouses located in Gaza are shown in Table 2. It can be seen that 11 insecticides are commonly used in the greenhouse for different insects, nematodes and mites control, with an application rate ranges from 1-2.5 kg/ha. Moreover, the frequency of application is different from one insecticide to another. The applied insecticides included Nema-cur, Methomyl and Vertimic. These insecticides have LD₅₀ values ranged from 6 mg/kg as in Nema-cur to 34 mg/kg as in Lannate (WHO, 2012) these insecticides are extremely toxic. Moreover, some other insecticides applied in greenhouses have higher LD₅₀ than mentioned above such as Pirate, Malathion, and Pyrethrine (Tomlin 2000). These applied insecticides included organophosphorus compounds (Ops) carbamate compounds, organo-chlorine compounds (OC), triazoles, triazines and others. Ops and carbamate compounds are the most toxic compounds and create many health problems around the globe. In addition fungicides with different chemical groups (diazole, and triazoles) were applied in greenhouses in Gaza. These fungicides control fungal and bacterial infestation of cucumber, melon and tomato such as *Botrytis cinerica*, *Oidium spp*, *Oidiopsis sicula*, *Alternaria cucumerina*, *Pseudoperonospora cubensis*. They have LD₅₀ values ranged from 700-5000 mg/kg and unsafe (WHO 2009). It can be seen the water solubility of the applied insecticides and fungicides ranging from 0.1-2000 mg/l with different K_{ow}, indicating different penetration potential through the human body (Table 2). However, pesticides with high K_{ow} values, have strong penetration potential to move throughout the body, whereas pesticides with low K_{ow} values have lower potential to move throughout the human body.

3.2 Occupational parameters

It appeared that 70% of the greenhouse farmers are male and 30% female. Both gender were in the age group of 20-30 years old. Female farmers have 9 years of education level, whereas male farmers have 12-14

years of education. Regardless to different gender and education level, it appears that a greenhouse is a family business. So far, the farmers indicated that they have less than 5 years working experience with pesticides. Moreover, 65% of them (90 farmers) indicated that insecticides are harmful to insects only, with no effect on human beings. In addition, 70% (97 farmers) of greenhouse farmers indicated that they did not use protective clothes, gloves, glasses or long shoes during pesticides application or any agricultural activity. So far, 25% of them (35 farmer) used gloves only during pesticides solution preparation. Majority of greenhouse farmers revealed that insecticides and fungicides were applied while the greenhouse is totally closed and the application rate was 1.5 folds higher than the recommended rate. They also indicated that pesticide spray processes were applied mechanically using high volume techniques at the sever cases of pest control whereas manual applications were occasionally applied by farmers using low volume technique. Farmers revealed that both techniques resulted in face and skin contamination. Some farmers (51%) revealed that their internal clothes have the same odor of pesticide solution at the end of working day.

3.3 Field toxicity symptoms.

Toxicity symptoms observed among greenhouse farmers during pesticides application are shown in Table 3. It can be seen that cholinergic symptoms such as dizziness (12.9%), headache (16.55%), nosia (18.71%), diarrhea (11.51%) and vomiting (17.99%), were observed among farmers during and/or after application of Pirate, Malathion, Cymbush, Pyrethrins and Dimethoate. So far, tremors (7.91%), convulsions (10.08%) and slight ataxia (4.32%) were less dominant toxicological symptoms, in all locations and observed after Nemacur, Vertimic, Methomyl and Smash applications which are considered as extremely toxic insecticides (WHO 2012). Application of fungicides after insecticides provided similar toxic symptoms whereas application of fungicides in the greenhouse without following insecticides application did not provide toxic symptoms as farmers revealed during interview.

3.4 Effect on ACHE activity

The status of ACHE activity of farmers applied insecticides in greenhouse is presented in Table 4. The data was classified to five groups according to the toxicity symptoms. Group 1 included cases got slight ataxia, their ACHE levels were severely inhibited (SI) and reached to 307-769 u/l; Group 2, included farmers got convulsions and their ACHE levels ranged between 770-2888 u/l and referred to as inhibited (I); Group 3 included cases got tremors, their ACHE levels were ranged between 3000-4982 u/l and referred to as moderate inhibited (MI) and; Group 4, included cases got vomiting, diarrhea, nozai, and their ACHE ranged between 5000-7000 u/l and referred as slightly inhibited enzyme (SLI), and Group 5, included farmers got Headache, Dizziness and their ACHE level ranged between 7001-9776 and refereed as less inhibited enzyme (LI) and control group included farmers and individuals did not have toxicity symptoms, their ACHE level ranged between 10000-18200 u/l and referred to as normal range (NR),

It can be seen that the severely inhibited cases (SI) included cases with tremors, convulsions and slight ataxia. Their percentage are 7.91; 10.08 and 4.32% respectively (Table 3). This indicates that application of pesticides under greenhouse conditions seriously exposing the farmers to large health risk. So far, the majority of farmers applying pesticides under greenhouse conditions got toxicity symptoms with less inhibited ACHE (Table 4).

Moreover, presentation of Box Plots (Figure 1) showed all cases were below normal range (NR) of ACHE activity. This indicates that application of insecticides and/or fungicides in greenhouses has sever toxicity to farmers.

3.5 Clinical symptoms of acute toxicology cases

Analysis of data from personal health records is shown in Tables 5-6. Data indicated that farmers were admitted in the intensive care unit of the hospitals, where clinical and biochemical investigations were done followed by atropine treatments. It can be seen that blood pressure (BP) of case 1-3 are nearly low compared to case 4-5 which are quite normal. Moreover, white blood cells (WBC) are above normal range in cases 1, 2, 3 and case 5 whereas the value is quite normal in case 4. This suggests that insecticides undergone antibody antigen reaction and raised the WBC in most cases. Moreover, a slight reduction in the RBC was observed in all cases except case 4. This might be the regular level of the cases regardless to poisonous status of person. Atropine treatment continued in all admitted cases (five cases) until the level of ACHE activity increased. Then biochemical investigation including (sugar, triglyceride, urea, uric acid, ALT and AST. Moreover, concentrations of Creat. urea and glucose are nearly within the range except case1 has high glucose contents. In addition, clinical symptoms of the five cases are nearly similar except higher magnitude in some case. However, the clinic symptoms such as pin point pupil's , sweaty and salivation are more dominant in case 2 and 3 than other cases. These observations are in agreement with ACHE status of these case (Table 5). These data

indicate cholinergic effects. Moreover, urination, lacrimation were similar to other cases indication of no-cholinergic effect or a side effect of toxicity.

3.6 Correlations among ACHE, AST, ALT and uric acid

Correlations among ACHE, AST, ALT and urea are shown in Table 7. It can be seen that elevated levels of Uric acid, AST and ALT were observed in the severely inhibited case (SI) of ACHE. This inverse relationships among them indicate an oxidation stress due to toxicity with insecticides. In addition the high level of uric acid suggests that insecticide or fungicide interact with protein synthesis or metabolic pathway. Figures 1-3, show distribution of cases by scattered Pox Plot distribution.

IV. Discussion

The presented results in Table (1) clearly shows the distribution of greenhouse in Gaza Strip and working farmers. It appeared that the total greenhouses are 57 that employed 139 farmers 70% of them are male. The applied insecticide or fungicides have different solubility limits in water, different K_{ow} , different vapor pressure (Henry constant), different application rate and frequency (Table 2). These data indicate that applied pesticides have different behavior under greenhouse conditions. Moreover, toxicity symptoms under greenhouse conditions (Table 3) showed cholinergic and non-cholinergic effects. The explanation of these results is that applied insecticides contained OP and oxim carbamate compounds that regard as strong acetylcholine esterase inhibitors as indicated by WHO (2009) and fungicides have high LD_{50} values, indicating non-cholinergic effects. Moreover, toxicity symptoms associated with ACHE levels (Table 4) indicated that farmers exposed to extreme toxic substances. The explanation of these results is that under greenhouse conditions, high temperature and humidity, farmers may be exposed to pesticides in different ways such as inhalation, ingestion and/or skin absorption. Moreover, farmers revealed that they used high and low volume techniques during pesticides spray process. Under high volume technique, farmers may ingest few drops of spray solution whereas at low volume technique farmers may become in contact with micro-droplets of pesticides active ingredients that penetrate the skin and adipose tissues according to K_{ow} and reach the active site in the body faster than usual and make toxic symptoms as in Table 3. These multi-exposure techniques may enhance the toxicity of pesticides. Moreover, application of insecticides followed by fungicides may expose farmers to binary mixture toxicity. This agrees with recent reports (El-Nahhal et al., 2015, 2016, El-Nahhal 2016; García-García et al., 2016; Ahmad et al., 2016; Araoud et al., 2016; Steerenberg et al., 2008; Riu et al., 2008;) who found decreased levels of acetyl cholinesterase in greenhouse workers due to pesticide exposure. Moreover, the data in Figure 1 clearly show that all tested cases are below the normal level of ACHE activity.

Moreover, the data from personal health records (Tables 5-6) are in agreement with ACHE activity determination (Table 4). These records agree with Aroonvilairat et al., (2015) who found similar observations. This harmony of results indicate consistency of work. Nevertheless, appearance of slight ataxia, severe toxicity cases (4.32%) and elevated levels of ALT, AST and urea might be an indicator of synergism. In addition, it has been shown the OP compounds have a direct effects on elevating ALT and AST levels (Table 7). Our results are in accord with Araoud et al., (2016) who found elevated ALP activity compared with untreated rats. More supports to our results come from Begum et al., (2015) who revealed that serum ALP, AST, ALT, and urea were increased significantly in chlorpyrifos treated birds. Moreover, Yang et al., (2012) reported oxidative damage in rat livers exposed to dichlorvos, acephate, dimethoate and phorate. These insecticides are used by greenhouse workers (Table 3).

However, low percentage of cases with elevated levels of ALT, AST and uric acid associated with slight ataxia, convulsions, and tremors may indicate uncertainty of these findings. Nevertheless, it can be suggested that greenhouse conditions enhance the evaporation of pesticides due to high temperature and exposing farmer lungs to toxic vapors. This situation might enhance appearance of oxidation stress accordingly elevated levels of liver enzymes were observed. Some authors found elevated liver enzyme in farmers worked in greenhouse condition. Moreover, recent published work (El-Nahhal 2016) found elevated levels of liver enzymes in farmers having long term exposure to pesticides. Nevertheless, exposure to insecticides under greenhouse condition elevated the oxidation stress in liver (Yang et al., (2012)) and result in elevation of ALT and AST levels. The data in Figure 1-3, indicate that the cases were significantly different from the control sample (non-farmers).

It can be suggested that irregular metabolic reaction of amino acid may occur resulting in accumulation of uric acid in the blood. Our results agree with Gaikwad et al., (2015) who indicated elevated level of uric acid among grape garden growers.

V. Conclusion

Application of insecticides and fungicides in greenhouse as individuals or following each other exposing farmers to acute toxicity that resulted in appearance of cholinergic symptoms such as Convulsions,

Tremors, Dizziness, Headache, Nozai, and Diarrhea. Acute toxicity was associated with severe reduction on ACHE activity and appearance of clinical symptoms such as pin point pupil's, sweaty and salivation. Moreover, elevation of AST ALP and uric acid concentration in blood serum were observed in farmers got slight ataxia. Frequent applications of insecticides followed by fungicides exposed farmers to binary and tertiary mixtures. Toxicity symptoms were highly potent under greenhouse conditions. Contentious exposure to pesticides under greenhouse condition may damage hepatic and nephron cells.

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Ethical Statement

This study was not funded by any organization

Compliance with Ethical Standards

Conflict of Interest: Author declares that he has no conflict of interest.

The study comply with the international ethics issues. Consent form was filled by each farmer participate with the study. Helsinki human right ethics were received before conducting the study.

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Table 1. Distribution of farmers using pesticides in different locations in Gaza Strip

Item	Greenhouses			
	Rafah	KHY	Gaza	North
Greenhouse	10	11	17	19
farmers	28	27	39	45
aNon-farmers	3	3	5	5

Table 2. List of insecticides and fungicides applied in greenhouses

Serial #	Pest	Pesticides	Rate g/d	Freq.	LD ₅₀ mg/kg	Sol. Mg/L	K _{ow} logP
1	Tetranychus urtica	¹ Smash(quinalphos)	150-200	57	71	17.8	4.44
		³ Pirate (chlorfenapyr)	40	18	441	Ins	4.83
2	Aphidoidea	¹ Malathion	200	66	1375	145	2.75
		⁴ Methomyl (lannate)	50	56	34	⁵ 57.9	0.093
3	Bemisia tabaci	Pyrethrins	60-100 ml		1030	0.2	5.9
4	Trips tabaci	¹ Nemacur (fenamiphos)	200	10	6	400	3.3
		⁴ Cymbush (cypermethrin)	150	37	250	0.004	6.6
5	Spodoptera littoralis	¹ Dursban (chlorpyrifos)	200	56	135-165	1.4	4.7
		Vertimic (abamectin)	150	29	10	7-10	4.4

		¹ Dimethoate	200	37	387	*23.3	0.704
6	Botrytis cinerea Oidium spp Oidiopsis sicula	Rovral (iprodione)	100	12	2000	13	3
		Antracol (2-naphthoxyacetic acid)	250	25	1000	-	-
7	Pseudoperonospora cubensis Pseudomonas syringae	Daconil (chlorothalonil)	150	25	5000	0.81	2.92
		Bayfidan (triadimenol)	50	100	700	33	3.28
8	Alternaria cucamerina	Copper sulfate (tribasic)	10	12	100	Ins	-

1= Organo-Phosphorus compounds; 2= Oxim carbamate compounds; 3= Bio-pesticides; 4= Pyrethroids; 5= Organo-Chlorine compounds; 6= Thiazoles; 7= Thiocarbamate; S= suspended, *= by inhalation, N=Nematicides

Table 3. Field toxicity symptoms among framers

Toxicity symptoms	Locations				Total	%
	Rafah	Kh Y	Gaza	North		
Dizziness	3	4	5	6	18	12.9
Headache	4	5	6	8	23	16.55
Nozai	5	6	8	7	26	18.71
Diarrhea	3	2	6	5	16	11.51
Vomiting	6	5	6	8	25	17.99
Tremors	3	3	2	3	11	7.91
Convulsions	3	1	4	6	14	10.08
Slight ataxia	1	1	2	2	6	4.32

Table 4. Acetyl cholinesterase activity among farmer having different symptoms.

Groups	Range (u/L)	Status of ACHE	Cases	
			Number	%
Farmers got slight ataxia,	307-769	SI	6	4.32
Farmers got convulsions	770-2888	I	14	10.07
Farmers got tremors	3000-4982	MI	11	7.91
Vomiting, Diarrhea, Nozai	5000-7000	SLI	67	48.20
Headache, Dizziness	7001-9776	LI	41	29.5
control group (not farmers)	10000-18200	NR	16	00

SI = Sever inhibition; I= Inhibition; MI= moderate inhibition; SLI= Slight inhibition; LI = less inhibition and NR = normal range .

Table 5. Acute toxicity and biochemical investigation of hospitalized cases

Measured item	Case 1	Case 2	Case 3	Case 4	Case 5
BP	90/70	75/60	80/60	120/70	110/70
WBC (10 ³ /mm ³)	22	11.5	12.5	7.2	15
RBC(g/dl)	4.74	4.74	5.76		5.4
Hb(g/dl)	11.3	12.2	13.9	14.2	11
PLT(10 ³ /mm ³)	307	508	307	262	270
Creat (mg/dl)	0.68	1.6	0.53	0.9	1.1
Urea (mg/dl)	36	35	29	28	25
GLU (mg/dl)	219	90	70	nm	Nm
Ca (mg/dl)	4.1	4.1	8.43	9.2	Nm
K (mg/dl)	0.36	0.36	0.41	0.18	Nm
ACHE (u\l)	2700	420	3897	3981	3500
ALT (u\l)	11	602	30	29	Nm
AST (u\l)	30	143	14	15	Nm

nm= not measured The data adopted from

Table 6. Clinical symptoms of acute toxicity cases

symptoms	Case 1	Case 2	Case 3	Case 4	Case 5
pin point pupil's	√	√√	√	√	√
Sweaty	√	√√	√√	√	√
Fusculation	√	-	-	√	√
Drowsiness	√	√	√√	√	-
Swelling	-	√	√	√	√
Salivation	√	√√√	√√	-	√
Urination	-	√	√	-	-
Lacrimation	√	√	√	√	√

√, √√ and √√√ indicate magnitude of symptom as evaluated by visual rating and refers to slightly affected, affected and severely affected respectively.

Table 7. Urea, AST and ALT levels among farmer having different symptoms.

Groups	Levels			Status of ACHE	Cases	
	Urea	AST	ALT		Number	%
Farmers got slight ataxia,	120-132	121-450	91-144	SI	6	4.03
Farmers got convulsions	101-120	91-120	71-90	I	7	4
Farmers got tremors	71-100	76-90	56-70	I	8	5
Vomiting, Diarrhea, Nozai ¹	45-70	46-75	41-55	SLI	78	49.66
Headache, Dizziness ²	31-44	36-45	38-40	LI	49	28.19
control group (not farmers)	15-30	4-35	9-37	NR	16	00

UA= uric acid ; SI = Sever inhibition; I= Inhibition; MI= moderate inhibition; LI= Slight inhibition; LI = less inhibition and NR = normal range . 1, and 2 indicates that the group received additional members from the other groups

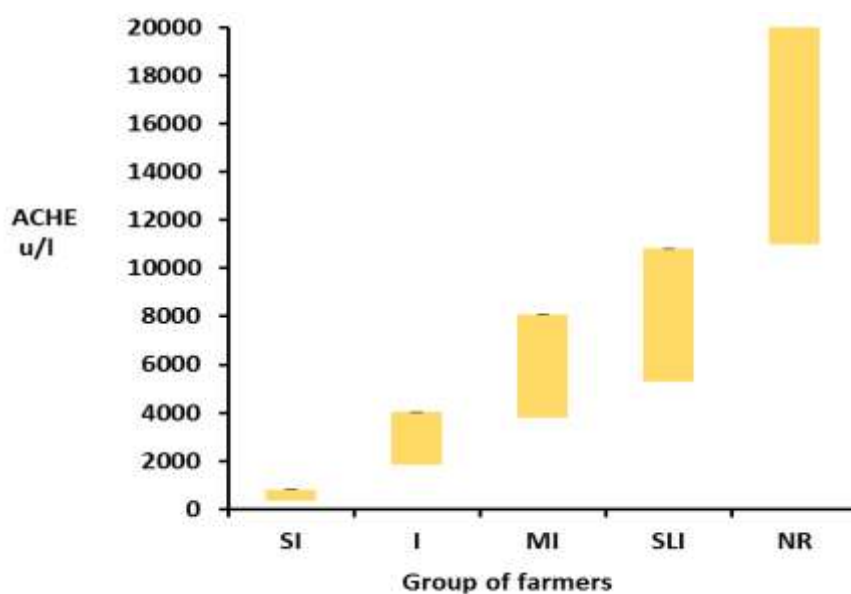


Figure 1. Distribution ACHE case among farmer group

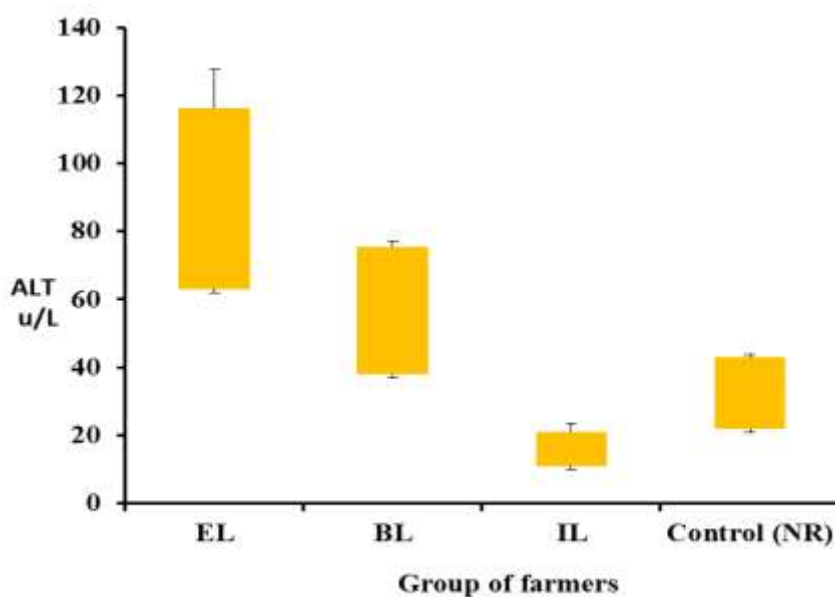


Figure 2. Distribution of ALT case among farmer group

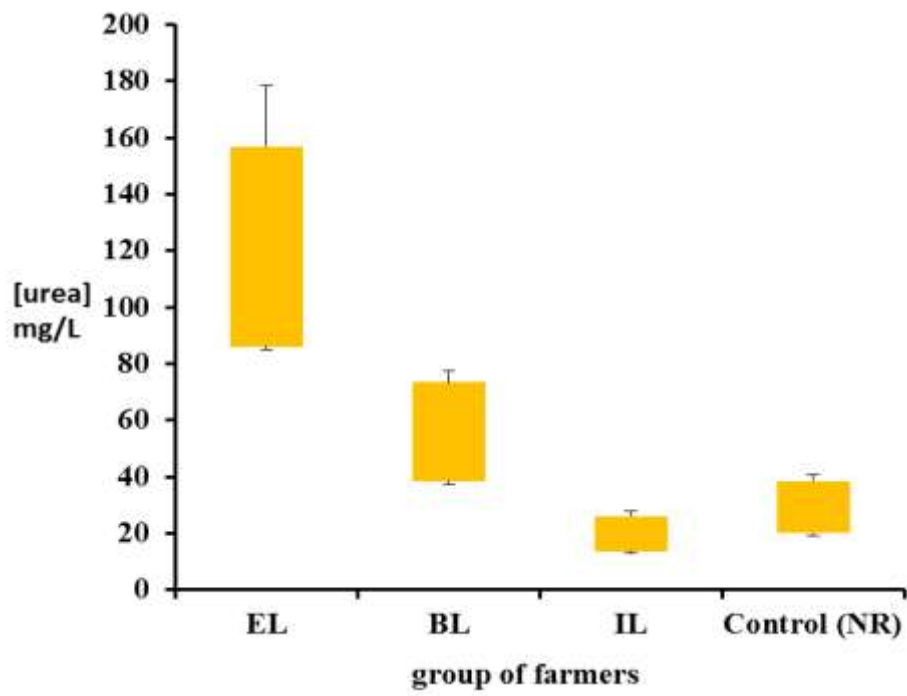


Figure 3. Distribution of urea conc among faramer group